First *Mariner Mos1* Transposase Inhibitors

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Abstract: We described chemical inhibitors of *Mos1* transposition. Some were already known to affect a related prokaryotic transposase (Tn*5*) or HIV-1 integrase, whereas the other were new compounds in this field. The new compounds were all organized around a bis-(heteroaryl)maleimides scaffold. Their mechanism of action depended on the chemical substitutions on the scaffold. The cross-activity, between HIV-1 integrase and *Mos1* transposase, of the new group of inhibitors showed that *Mos1* transposase could constitute an excellent surrogate HIV-1 inhibitor screen.

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Key Words: Heterocyclic chemistry, maleimides, transposon inhibitor, HIV-1 integrase inhibitor, mariner inhibitor.

INTRODUCTION

DNA transposons are Class II transposable elements that correspond to discrete DNA segments that are "naturally" able to move within genomes (for a review see [1]). In addition, some of them have been shown experimentally to have a wide-ranging capacity to move into species distantly related to the host from which they had initially been isolated. These properties have made it possible to develop transposon tools for insertional mutagenesis and germ-line transgenesis in model organisms, and potentially for gene therapy. *Mos1* [2], *Himar1* [3], *Minos* [4] *PiggyBac* [5], *Sleeping Beauty* [6], and *Tol2* [7] were selected as the main candidates for the development of such transposon tools.

Mos1 is a 1286-bp long element, which is terminated at both extremities by 28-bp inverted terminal repeats (ITRs) that are imperfectly conserved in sequence. *Mos1* contains a single open reading frame coding MOS1, a 345-amino acid transposase, and it is known to move within the genome of its hosts using a cut-and-paste mechanism. We hypothesize that this mechanism could well consist of four main steps: [1] MOS1 homo-dimerization, [2] assembly of the synaptic complex, [3] excision of *Mos1*, and [4] target recognition and insertion at a new locus (supplemental data, Fig. (**S1**)). Although the organization and composition of the *Mos1* synaptic complex are still debated [8,9], a complex consisting of a pair of *Mos1* ITRs and a transposase tetramer (*i.e.* Paired End Complex 2: PEC2) is probably the complex that carries the activities that allow the concerted integration of the transposon at the new locus [8]. These activities rely on a well-conserved triad of amino acids, DD34D, that together coordinate the cationic cofactors required for catalysis [10]. The organization of its catalytic core links MOS1 to a large family of transposases and integrases that share a similar catalytic core, known as the RNase H-like core [11]. Crystallographic studies have shown that despite the lack of sequence similarity, the structure of the catalytic core, including the location of the invariable DDE/D residues, is very similar in three transposases (MuA, Tn*5* and MOS1) [10,12], and two integrases (HIV-1 and RSV) [12-14]. Due to the presence of this highly conserved triad, these proteins have been designated "DDE-transposases". In fact the *mariner* transposases are an exception, and have a DDD triad [12,13]. DDE-transposases carry out phosphoryl transfer reactions using the same basic chemical steps and similar active sites [11]. Several bacterial transposons, such as *Mu*, simply nick and transfer the 3' ends of the transposon to the target [15]. Transposons of the IS*3* family perform an asymmetric nick at just one 3' end of the element, followed by a strand transfer immediately outside the other transposon end [16]. In both *Mu* and IS*3*, transposition requires DNA replication. Several bacterial transposons, such as Tn*5* and Tn*10*, nick and transfer the 3' ends of the transposon to the complementary strand, thus forming hairpins on the transposon ends [17,18]. In contrast, several eukaryotic transposons, such as Ac/Ds, nick the 5' ends of the transposon, and transfer the 3' ends of the flanking DNA to the complementary strand, thus forming hairpins on the flanking DNA [1]. Other eukaryotic transposons, such as *mariner*, perform a double strand DNA cleavage at the transposon ends without forming a hairpin, and transfer the 3' ends of the transposon to the target DNA [19]. Finally, the DDE-integrase of retro-transposons and retroviruses performs the integration steps using strand transfer reactions that look like those performed by the "cut and paste" DDE-transposases of the *mariner* family.

In order to improve our fundamental understanding of MOS1 activities, but also to provide a better way of controlling transposition in the context of transposon tools, it will be useful to have compounds that are able to inhibit transposase activities. To date, no such compounds have been avail-

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able for eukaryotic transposases. The aim of the work reported here was to isolate and characterize the first chemical inhibitors of a *mariner* transposase. 159 chemical compounds were selected, and their ability to inhibit *Mos1* transposition *in vitro* was assessed. Since it had recently been demonstrated that Tn*5* transposase can be used to characterize inhibitors of HIV-1 integrase [20,21], we included inhibitors of these two proteins in our panel, and tested their activity as MOS1 inhibitors. The other compounds were selected because they shared structural and chemical properties with established HIV-1 integrase inhibitors. Among the ten chemicals identified as being the best *Mos1* inhibitors, six were already known to be inhibitors of Tn5 transposase and/or HIV-1 integrase. The other four constituted a new group of drugs, and they all contained a bis-(heteroaryl) maleimides scaffold. The mechanism of action of these new substances depends on the chemical substitutions on the scaffold. First, the compounds that are *N*-substituted inhibited protein-DNA complex assembly more efficiently than the compound that is not. Second, the compound that contains formyl groups (instead of the acid functions) more efficiently prevented integration (the last step in transposition) than the other compounds. We also provide evidence that bis-(heteroaryl) maleimide derivatives are useful inhibitors of HIV-1 integrase. This cross-reactivity (between HIV-1 integrase and *Mos1* transposase) of the new inhibitors once again confirms the close relationships that exist between the DDE-enzymes.

IDENTIFICATION OF MOS1 INHIBITORS

In order to identify inhibitors of *Mos1* transposase, we screened a collection of 159 molecules. The first part of the panel (29 compounds) that we tested consisted of inhibitors of Tn*5* transposase and/or HIV-1 integrase (for details see **S3**, experimental section in supplemental data). The other compounds consisted of substituted-heterocyclic derivatives. Although these compounds had never been tested as inhibitors of an integrase or transposase, they were selected because they shared structural and chemical properties with a previously characterized HIV-1 integrase inhibitor.

A transposition assay was performed using purified MOS1 protein fused to the MBP, and the pBC-3T3 plasmid carrying a pseudo-*Mos1* transposon to screen the collection of chemicals. The pseudo transposon was formed by two 3'ITRs and the tetracycline resistance gene, without promoter. The same plasmid was used both as the donor of pseudo-*Mos1*, and as the target for transposon integration: MOS1 excises a 3T3 from pCB-3T3, and then triggers the reinsertion of this pseudo-*Mos1* within the CAT gene of another pBC-3T3 molecule. Here, the *in-vitro* assay is carried out in the same way as had previously been found for the bacterial assay (*29*). Thus, in this assay, transposition events were revealed by promoter tagging, the tetracycline resistance being activated through the CAT gene promoter.

The concentration of compound used for the initial screen was 80 μM. 116 of the 159 compounds tested had an Inhibition Factor (IF) of less than 5, compared to the control experiment performed in the absence of inhibitor but in the presence of DMSO, and 33 had an IF of between 5 and 25 (80% < inhibition < 96%). Among the 29 compounds previously identified as HIV-1 integrase and/or Tn*5* transposase inhibitors, 25 were found in these groups (IF<25) (Fig. (**1a**)). The corresponding transposition frequencies are given as Supplemental data (Table (**S1**)).

The ten best compounds had an IF of greater than 25 (> 96% of inhibition). The structures of these inhibitors are shown in Fig. (**1b**). Compounds (**1**) to (**4**) were bis-(heteroaryl)maleimide derivatives, constituting hitherto-unknown inhibitors of DDE/D transposases. Compounds (**5**) and (**6**) were *N*-methylpyrrole polyamides, which are known to interact with DNA (review in [22]). Compound (**7**) was a biscoumarin derivative that was also shown to be an inhibitor of Tn*5* transposase and HIV1-integrase [20]. Compounds (**8**), (**9**) and (**10**) were a cinnamoyl derivative, a benzoic acid derivative and a thioxothiazolidin substituted by carboxylic acid, respectively. These three compounds had already been identified as Tn*5* transposase inhibitors [20]

Inhibitory Activity on Mos1 Excision

To further characterize the inhibitory activity of these ten compounds and their mechanisms of action on *Mos1* transposition, the ability of each chemical to inhibit transposon excision was tested *in vitro*, using pBC-3T3 as the transposon donor. MOS1 triggers the transposon excision from the plasmid, producing two linear DNA fragments, the transposon (T=1.2 kb) and the plasmid backbone (B=3.4 kb). After excision, the transposon can be reinserted into a target. The excision activity of the drug was measured by quantifying the plasmid backbone, because this is an end product of the reaction. As an example, the assay performed with compound (**9**) is shown in Fig. (**2a**). In the absence of drug, the transposase released the transposon from the donor plasmid (lane 8). At the highest concentration of drug (1 mM), excision of the transposon was completely abolished (lane 2). To determine the concentration of drug required to inhibit 50% of the reaction (IC50), the percentages of inhibition measured from two independent experiments were plotted against the concentration of drug. Similar experiments were performed with the other nine chemicals, and the corresponding IC50 values were estimated (Fig. (**2b**)). Compounds (**2**) to (**6**) were the most effective inhibitors of excision, with IC50 values around 10 μM (Table **1**). A second panel of drugs, with IC50 values ranging from 35 μM (compounds (**1**) and (**10**)) to 150 μM (compound (**9**)), included the Tn*5* inhibitors.

Our data indicated that all the inhibitors have a significant impact on the excision of the transposon. Because the Tn*5* transposase inhibitors (compounds (**7**) to (**10**)) blocked formation of the Tn*5* synaptic complex [20], and because compounds (**5**) and (**6**) were powerful DNA binding protein inhibitors (review in [22]), we decided to try to find out whether these six compounds act at the level of the formation of transposase-ITR complexes, a step that takes place before excision.

Inhibitory Effect on the Assembly of MOS-ITR Complexes

We first focused our attention on the ability of drugs 5 to 10 to inhibit the formation of complexes between MOS1 and the 3'ITR [8], using a gel shift mobility assay (EMSA). MOS1-ITR complexes were formed with a radiolabeled

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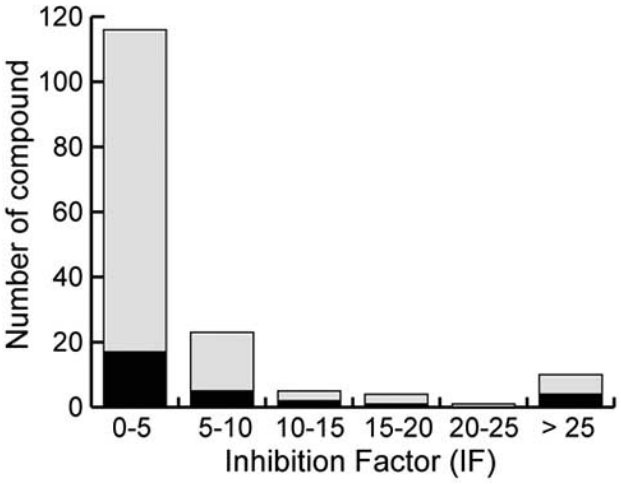

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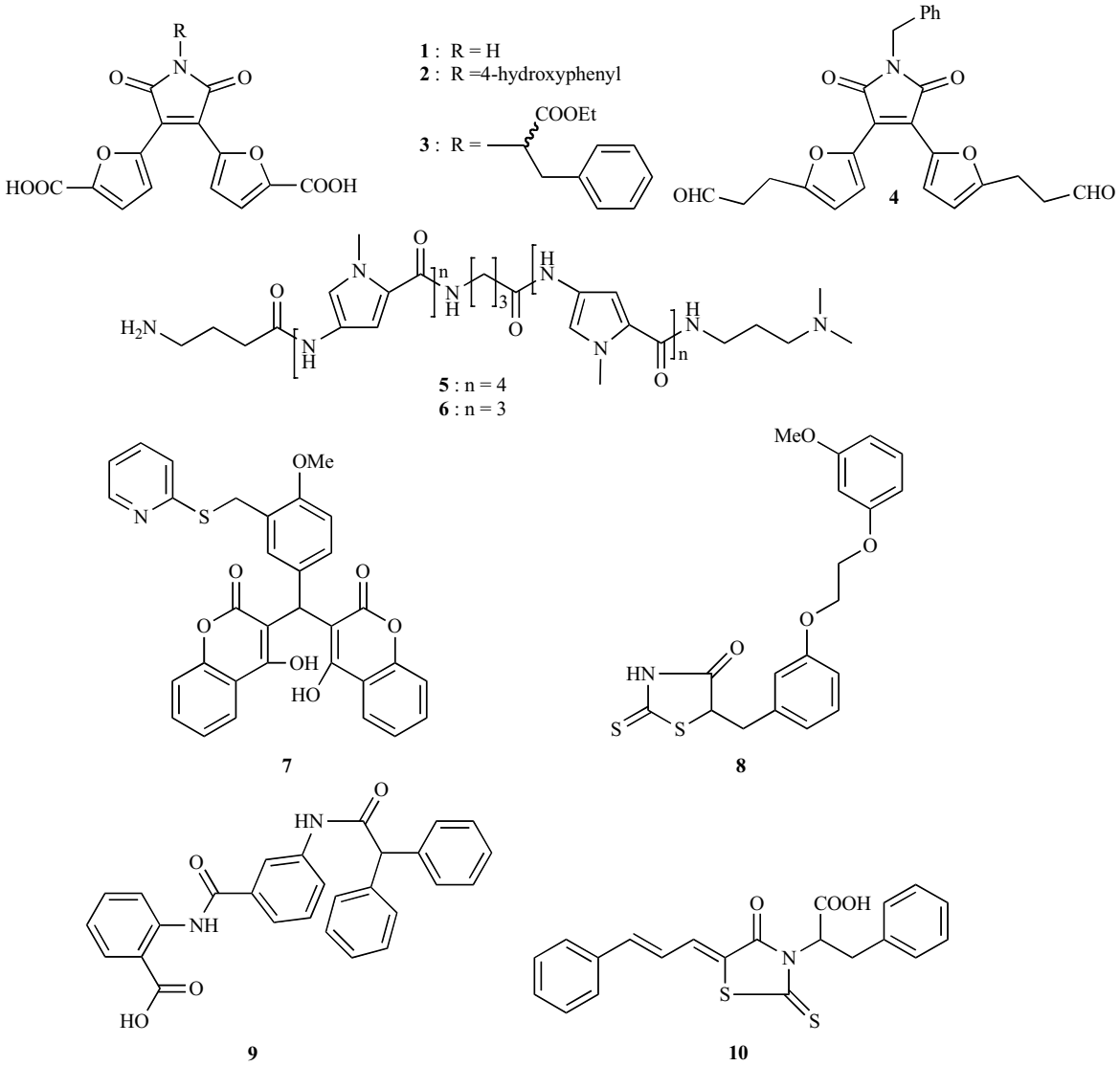

Fig. (1). Inhibitory activity of the 159 compounds. **(a)** Inhibition Factor of the 159 chemicals screened: each compound was tested at a concentration of 80 µM in a transposition assay. The inhibition factors (IFs), which correspond to the ratio between transposition frequency of the control reaction performed in the absence of the chemical, and the transposition frequency of the reaction in the presence of 80 µM of compound, are reported in the histogram. Chemicals previously identified as potent Tn*5* transposase or HIV-1 integrase inhibitors are shown in black. **(b)** Chemical structures of the ten compounds with IF > 25.

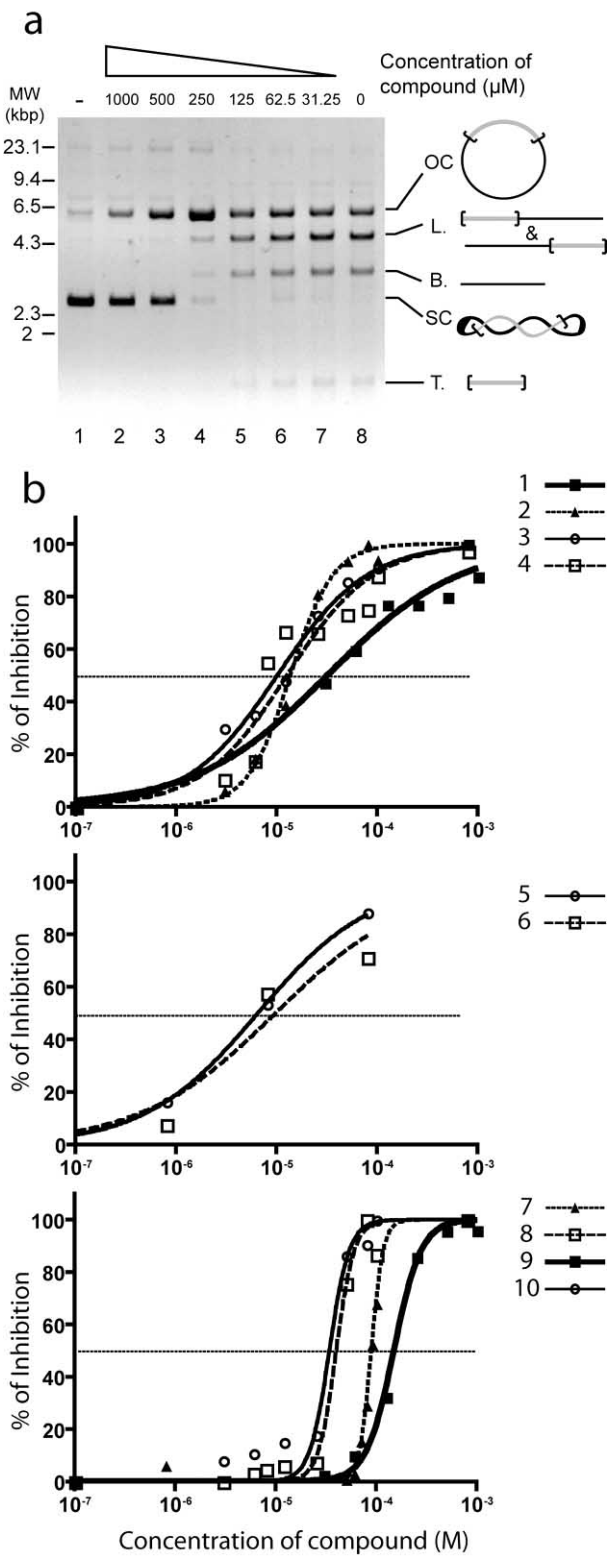

Fig. (2). Inhibition of transposon excision. **(a)** Inhibitory activity of compound (**9**) against transposon excision. The super-coiled pBC-3T3 plasmid (SC) is used as a transposon donor (lane 1). In the presence of transposase (lane 8), a first strand nicking reaction relaxed the donor plasmid (OC product). Second strand cleavage linearized the donor plasmid (L. product). Cleavage at both ITRs led to the excision of the transposon, releasing the transposon (T. product) and the plasmid backbone (B. product). The intermediate and final products of the reaction were visualized by electrophoresis on agarose gel and ethidium bromide staining. The reaction was performed in the absence of transposase (lane 1), without the inhibitor (lane 8) or with decreasing concentrations of compound (**9**) (lanes 2 to 7). The released backbone was quantified as an end product of the transposon excision reaction. **(b)** Inhibitory activity of the compounds. The activity of the ten compounds was plotted against compound concentration, and fitted on a sigmoid dose-response curve using GraphPad Prism software. First panel; compounds: (**1**) (black square), (**2**) (black triangle), (**3**) (open circle) and (**4**) (open square). Second panel; compounds: (**5**) (open circle) and (**6**) (open square). Third panel; compounds: (**7**) (dark triangle), (**8**) (open square), (**9**) (black square) and (**10**) (open circle).

Table 1. Effect of Each Inhibitor on *Mariner Mos1* Excision. IC50 Values for Excision (Exc.) are Reported in μM

Compound	IC50 Exc. (μM)	95% Confidence Intervals
1	33	18 < IC50<54
2	14	12 < IC50<15
3	10	7 < IC50<13
4	12	9 < IC50<18
5	6	5 < IC50<12
6	10	4 < IC50<22
7	89	86 < IC50<91
8	40	36< IC50<44
9	152	138 < IC50<166
10	34	29 < IC50<39

3'ITR (ITR70) in the presence of a concentration of drug equivalent to five-times the IC50 excision value, calculated for each compound. Our data (Fig. (**3a**)) indicated that the assembly of these complexes was almost totally prevented in the presence of compounds (**5**) to (**9**), and considerably inhibited (50% inhibition) in the presence of compound (**10**). The data observed here for compounds (**5**) and (**6**) are consistent with what is known about the mechanism of action of *N*-methylpyrrole polyamides [22]. These compounds are able to interact with DNA, and the slight modification in the migration pattern of the free probe (ITR70*, see Fig. (**3a**)) in the presence of these two compounds may account for this effect. Thus, compounds (**5**) and (**6**) targeted the DNA, and probably competed with MOS1 to bind to the ITR. Our data for compounds (**7**) to (**9**) are also consistent with the known mechanism of action of these drugs on the assembly of the Tn*5* transposase-ITR complex. Indeed, Ason *et al*. had previously shown (using EMSAs) that these compounds inhibit Tn*5* complex assembly, with IC50 values ranging from 5 to 45 μM [20]. Even though compound (**10**) efficiently inhibited Tn*5* transposase-ITR complex assembly, this compound seemed to have little impact on the assembly of MOS1-ITR complexes (see Fig. (**3a**)).

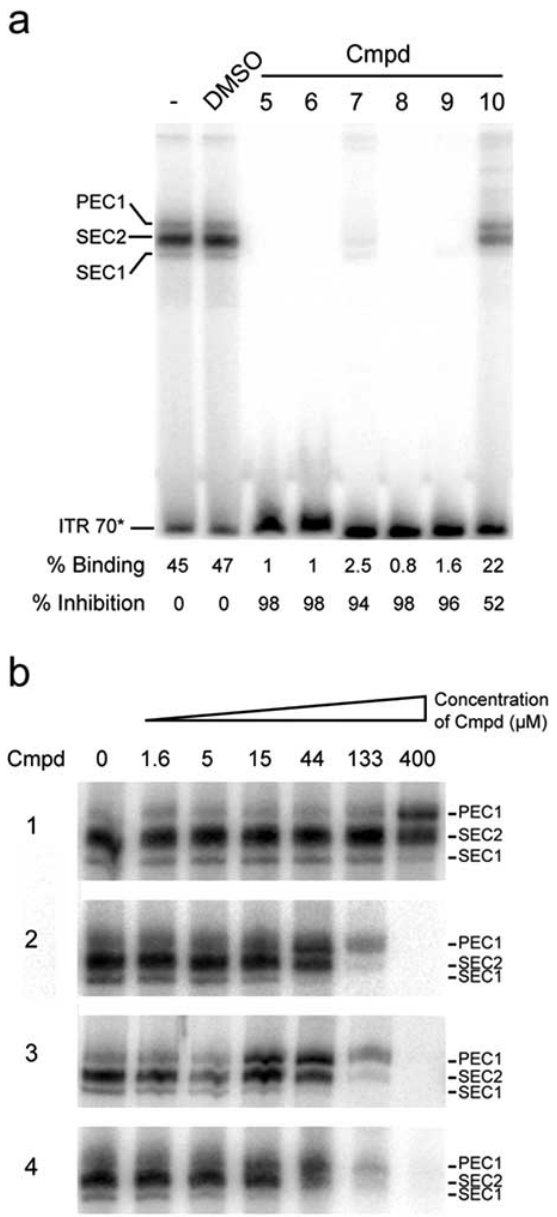

Fig. (3). Impact of the inhibitors on the assembly of MOS-ITR complexes. EMSAs were performed with 400 nM MBP-MOS1, 5 nM ITR70 as a probe and the 10 compounds (**1** to **10**) as indicated. **(a)** Compounds (**5**) to (**10**). (-): without compound; D: DMSO. The compounds are indicated at the top of the figure. They were used in concentrations corresponding to 5 times the IC50 reported in Table (**1**). Briefly, these concentrations were: 65 μM (compounds **(5)** and (**6**)), 440 μM (compound (**7**)), 200 μM (compound (**8**)), 750 μM (compound (**9**)) and 170 μM (compound (**10**)). The complexes observed and the unbound DNA (ITR70*) are indicated. SEC1 (Single end complex 1), SEC2 (Single end complex 2) and PEC1 (Paired end complex 1) were already described [8]. Percentage binding measured from the percentage of probe retained in the three complexes observed. Percentage inhibition measured as the loss of binding in the presence of the compounds. **(b)** Compounds (**1**) to (**4**). Accurate analyses of compounds (**1**) to (**4**)were performed using different concentrations of drugs ranging from 1.6 to 400 μM, as indicated at the top of the figure. The corresponding compounds are indicated on the left. Only complexes are shown and identified on the right (as in (**a**)).

As our findings for compounds (**5**) to (**10**) matched the published mechanism of action of these drugs, we focused our attention on the new drugs. Before doing any further investigations of the new transposase inhibitors (drugs (**1**) to (**4**)), we first checked to see whether these compounds are directed against MOS1, but not against the DNA (like compounds (**5**) and (**6**)). This was done either directly, testing the direct binding of the compounds to the ITR in electrophoretic mobility shift assay (EMSA) or indirectly using excision assays with large excess of competitor DNA (see supplemental data, Fig. (**S2**)). Our data showed that compounds (**1**) to (**4**) did not interact with the DNA.

We therefore assayed the ability of the new maleimide derivates to inhibit the formation of complexes between MOS1 and the 3'ITR. MOS1-ITR complexes were formed with a radiolabeled 3'ITR (ITR70) in the presence of drug concentrations ranging from 0 to 400 μM, and analyzed using EMSA (Fig. (**3b**)). Our data therefore indicated that compound (**1**) modified the ratio of MOS1-ITR complexes at a high drug concentration (400 μM, lane 7), leading to enrichment in PEC1 (paired end complex 1) to the detriment of SEC2 (single end complex 2). The other compounds acted similarly, modifying the ratio of MOS1-ITR complexes, but for drug concentrations ranging from 15 μM (compound (**3**), lane 4) to 44 μM (compounds (**2**) and (**4**), lane 5). At higher concentrations, compounds (**2**) to (**4**) were able not only to modify the ratio, but also to prevent the assembly of MOS1-ITR complexes (lanes 6 and 7). These data paralleled the IC50 excision values, which indicated that compounds (**2**), (**3**) and (**4**) were more efficient inhibitors of *Mos1* excision than compound (**1**).

As we think that MOS1 dimers are formed prior to ITR binding [23], we assayed drugs (**1**) to (**4**) to find out whether they were able to inhibit the formation of such dimers. Using a truncated form of MOS1 (Tnp[1-85]) that contains the MOS1 dimerization sub-domain, we measured MOS1 dimerization in the presence of compounds (**1**) to (**4**) by means of glutaraldehyde fixation experiments, as described [23]. None of the compounds inhibited the formation of Tnp[1-85] dimer at the 500-μM concentration (data not shown), which ruled out the possibility that these compounds could inhibit complex formation by destabilizing the N-terminal dimerization domain.

Taken together, these data indicated that these new inhibitors interacted with MOS1. This interaction could modify the organization of MOS1-ITR complexes, thus inhibiting *Mos1* excision, a subsequent step in the transposition reaction.

Effect of the New Inhibitors on Strand Transfer Reactions

Since excision and the strand transfer reaction are closely related reactions, and since strand transfer is a later step in the transposition reaction, we tested the new compounds to see whether they also inhibited the integration. Pre-cleaved ITRs were labeled on the 5' of the transfer strand, and used to form the integration complex. The inhibitor was added before the MOS1-ITR complexes had formed. ITR integration events in a target plasmid were detected after resolving

the reaction products on an agarose gel, as exemplified for compound (**1**), in Fig. (**4a**). One product, which co-migrated with linear forms of the target plasmid, was detected in the presence of both MOS1 and the catalytic cofactor Mg^{2+} (lane 7). The percentage inhibition produced by the new compounds was determined in four independent experiments, and plotted against the drug concentration (Fig. (**4b**)). Compounds (**1**), (**2**) and (**3**) inhibited the ITR integration, with IC50 values similar to those for excision (Table (**2**)), whereas compound (**4**) inhibited the strand transfer reaction 15-times more efficiently than excision. This indicated that compound (**4**) had some additional activity (when compared to compounds (**1**) to (**3**)) that might consist either of the inhibition of target capture or the direct inhibition of strand transfer catalysis, the two main steps of integration.

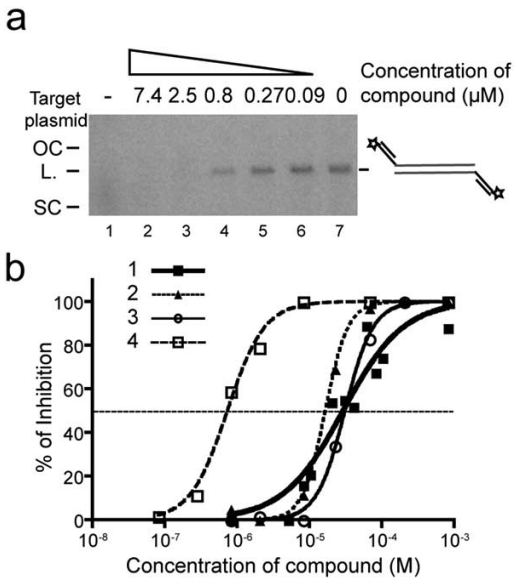

Fig. (4). Impact of the inhibitors on MOS1 strand transfer reactions. **(a)** *In-vitro* strand transfer inhibition activity of compound (**4**). Reactions were performed with pre-cleaved ITR substrate (radioactively labeled on the 5' extremity of the transferred strand). The pBC-KS vector was used as a super coiled plasmid target (SC). Reactions were stopped with EDTA, heated for 10 min at 75°C to disrupt the integration complex, and electrophoresed on a standard TBE-buffered 1% agarose gel. Concerted integration of transposon ends (two ITR) at the target site yielded a linear product (L.) as expected, whereas hemi-integration of transposon end (a single ITR) would have given a relaxed target plasmid (OC). After electrophoresis, the gel was stained for total DNA with ethidium bromide, dried and the radioactive products were recorded on a PhosphorImager. Lane 1; no transposase, lanes 2 to 6, decreasing concentrations of compound (**4**), lane 7; no inhibitor. **(b)** Inhibitory activity of compounds (**1**) to (**4**). The activity of compounds (**1**) to (**4**) (as measured in (**a**)) were plotted against the compound concentration and fitted on a sigmoid dose-response curve using GraphPad Prism software. Compounds: (**1**) (black square), (**2**) (black triangle), (**3**) (open circle) and (**4**) (open square).

Effect of the Inhibitors on HIV-1 Integrase 3' Processing and Strand Transfer Activities

We showed that Tn*5* and HIV-1 integrase inhibitors could also inhibit MOS1. To find out whether the new group of inhibitors were also able to block HIV-1 integrase, their activity was tested against the 3' processing and strand transfer activities of HIV-1 integrase. The assay was performed as previously described [24] using purified recombinant integrase and short radiolabeled oligonucleotide substrates, and the products were resolved on a denaturing urea gel. A typical assay, monitored using compound (**4**), is shown in (Fig. (**5a**)). Similar experiments were done using compounds (**1**) to (**3**), and the inhibition percentages found in three independent experiments were plotted against the drug concentrations (Fig. (**5b**) and (**5c**)). IC50 values for 3' processing, and strand transfer activities were calculated, and are reported in Table (**3**). Compounds (**2**) to (**4**) were efficient against both strand transfer and 3' processing. In contrast to compounds (**2**) and (**3**), compound (**4**) was significantly more efficient (5-times) against strand transfer activity (with an IC50 value of 5.5 μM) than against 3' processing (with an IC50 value of 29 μM). This data looks similar to what we observed using compound (**4**) against MOS1: integration was inhibited 15-times more efficiently than excision (see Tables (**1**) and (**2**)). Finally, compound (**1**) was the least effective against both HIV-1 integrase 3' processing and strand transfer activities. This effect, which had already been observed in MOS1 inhibition studies, was markedly enhanced for HIV-1 integrase.

Table 2. Effect of the New Inhibitors on *Mariner Mos1* Integration. IC50 Values for ITR Integration (Int.) are Reported in μM

Compound	IC50 Int. (μM)	95% Confidence Intervals
1	30	19 < IC50<45
2	16	15 < IC50<18
3	30	27 < IC50<34
4	0.7	0.6 < IC50<0.9

In conclusion, the bis(furanyl)*N*-maleimide derivatives that we have described as MOS1 inhibitors also have similar effects against HIV-1 integrase, demonstrating that this new group of inhibitors have cross activity against *Mos1* transposase and HIV1-integrase. As a consequence, MOS1 can be considered to be a new surrogate model for identifying HIV-1 integrase inhibitors.

CONCLUSIONS

The availability of specific inhibitors is very important for the analysis and control of elaborate mechanisms, such as transposition. For the first time, we have characterized chemicals that inhibit the transposition of *mariner*, the most widespread transposable element (TE) in eukaryotes. We used EMSA, excision and strand transfer assays to study in greater detail the mechanism of action of the ten most effective inhibitors identified in a panel of 159 compounds.

Tn5 and HIV-1 Integrase Inhibitors are Active Against MOS1

A first group of compounds consisted of compounds that had previously been identified as inhibitors of transposase

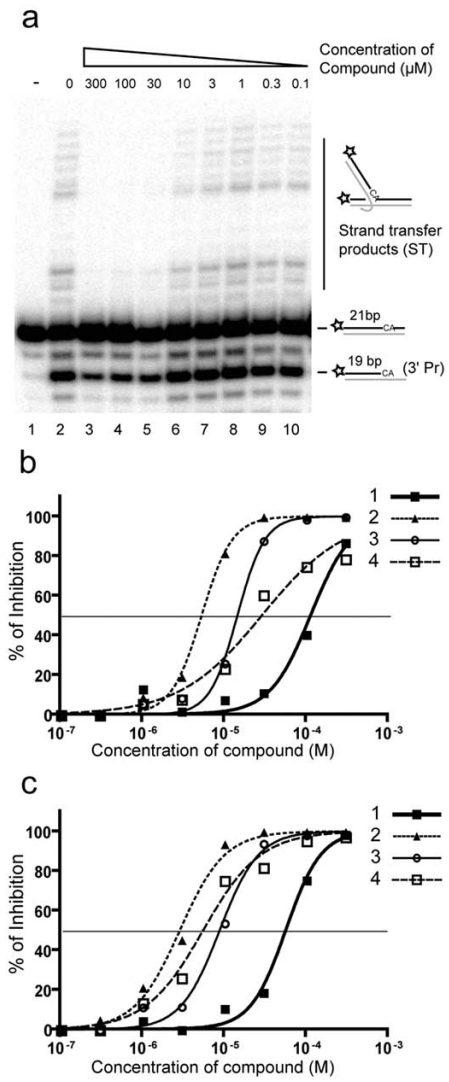

Fig. (5). Inhibitory activity of compound (4) on HIV-1 integrase 3' processing and strand transfer activities. **(a)** HIV-1 integrase inhibitory activity of compound (**4**): Assays were performed using purified recombinant HIV-1 integrase and a duplex oligonucleotide mimicking the U5 LTR extremity radioactively labeled on the 5' extremity of the processed strand. The reactions were stopped with EDTA, and electrophoresed on 18% acrylamide, 7M urea denaturing gel. 3' processing reaction yields a 19-mer product that is a substrate for the strand transfer reaction. Lane 1; no integrase, Lane 2; no inhibitor, lanes 3 to 10; decreasing concentration of compound (**4**). **(b)** 3' processing inhibitory activity of the compounds. The activity of the four compounds (**1** to **4**), from three independent experiments, was plotted against compound concentration, and fitted on a sigmoid dose-response curve using GraphPad Prism software. Compounds: (**1**) (black square), (**2**) (black triangle), (**3**) (open circle) and (**4**) (open square). **(c)** Strand transfer inhibitory activity of the compounds. The activity of the four compounds (**1** to **4**), from three independent experiments, was plotted against compound concentration, and fitted on a sigmoid dose-response curve using GraphPad Prism software. Compounds: (**1**) (black square), (**2**) (black triangle), (**3**) (open circle) and (**4**) (open square).

(Tn*5*) or integrase (HIV-1). Drugs 7 to 10 displayed a similar mechanism of action *versus* both MOS1 and Tn*5* transposase. They inhibited the formation of the complexes, probably by interfering with one of the DNA-recognition domains of the transposase. The Tn5 inhibitors were less effective against *Mos1* transposition, which may reflect differences between the mechanisms of DNA recognition and complex organization.

The target of the drugs allows us to distinguish between compounds (**5**) and (**6**) on the one hand, which bind DNA, and on the other, the other compounds, which are devoid of DNA-binding ability. Compounds (**5**) and (**6**) are hairpin polyamides, composed of a *N*-methylpyrrole polyamide connected by a γ-aminobutyric acid (γ-turn) linker, and bound to the minor groove of DNA [22]. They specifically interact with a short sequence of three or four A/T pairs for compound (**5**) and (**6**) respectively. These sequences are located at the tip, and also in the ITR substrate, which accounts for the effect of compounds (**5**) and (**6**) on *Mos1* transposition.

bis-(heteroaryl)maleimide Derivatives Constitute a New Scaffold of DDE-Enzyme Inhibitors

The panel of drugs that we tested as MOS1 inhibitors consisted mainly of hydroxyl-substituted heterocyclic molecules. Small molecules of this kind had already been identified as efficient inhibitors of HIV-1 integrase [25]. Nineteen of the compounds analyzed here were bis-(heteroaryl)maleimide derivatives. The most effective inhibitors we found were all organized around this scaffold (compounds (**1**) to (**4**)). However, scaffolds with no substituents were inefficient, suggesting that the bis-(heteroaryl)maleimide backbone itself is not the pharmacophore (data not shown). Maleimide *N*-substitution and heteroaryl substitution both had a major impact on both the mechanism of action and efficiency of the drugs. Here, we describe three examples. (i) Compound (**1**), which is not *N*-substituted, does not inhibit MOS1-ITR complex assembly, although differences in complexes ratio suggest that compound (**1**) may change the structure of the complexes, favouring PEC1 assembly. This compound has a similar effect against both the excision and strand transfer activities, and has slightly higher IC50 values than compounds (**2**), (**3**) and (**4**). (ii) Compounds (**2**) and (**3**), which are *N*-substituted, inhibit MOS1-ITR complex assembly more efficiently than compound (**1**). These compounds have a similar effect against both the excision and strand transfer activities. (iii) Compound (**4**), which is also *N*-substituted, but contains a formyl group (instead of the acid functions in compounds (**1**) to (**3**)) has similar activity against MOS1-ITR complex assembly and excision as compounds (**2**) and (**3**). Unlike compounds (**1**) to (**3**), compound (**4**) is 15-times more active against strand transfer activity than against *Mos1* excision.

Active bis-(heteroaryl)maleimide derivatives do not bind to DNA (this study and [26]), but they probably target the transposase or transposase-DNA complexes, with greater or lesser efficiency. There are (at least) two possible explanations for the differing efficiencies displayed by compound (**1**) and the other maleimide derivatives. First, the drug targets may differ as a result of the aromatic group substitution on the *N*-maleimide (compounds (**2**) to (**4**)). Second, all four compounds could have a similar target, but the bulky aromatic *N*-maleimide substitution may interfere with DNA

Table 3. Effect of the New Inhibitors on HIV-1 Integration. IC50 Values for 3' Processing (Proc.) and Strand Transfer (StT.) are Reported in μM

Compound	IC50 Proc. (μM)		IC50 StT. (μM)	
1	116	59 < IC50<228	58	35 < IC50<94
2	5.3	4.6 < IC50<6.1	3	2.3 < IC50<3.6
3	15	10 < IC50<19	9	6 < IC50<13
4	29	14 < IC50<60	5.5	4 < IC50<7.7

binding, whereas the non-substituted compound tolerates the presence of the DNA. Further studies are required to identify the exact target(s) and mechanism of action of this family of compounds.

Similarly, three situations were observed in the case of HIV-1 integrase inhibition. (i) Compound (**1**), which is not *N*-substituted, had higher IC50 values than compounds (**2**), (**3**) and (**4**). In addition, this compound yielded more variable data, with a wide 95% confidence interval: for instance the IC50 for 3' processing was (59-228) μM. This point was also confirmed (albeit to a lesser extent) against MOS1. This raises the question of the solubility of non-*N*-substituted compounds. (ii) Compounds (**2**) and (**3**), which are *N*-substituted, display similar activity against both the excision and the strand transfer activities, with very good 95% confidence intervals. (iii) Compound (**4**), which is also *N*-substituted but contains a formyl group, is 5 times more active against strand transfer activity than against 3' processing activity.

The effect of compound (**4**) on the final step in *Mos1* transposition and/or HIV-1 integration is very interesting. Indeed, the data presented here suggest that compound (**4**) prevents the integration step in both systems. During both transposition and retroviral integration, this step involves at least two phases: (i) capturing the DNA targeted for integration and (ii) the strand transfer reaction itself. Compound (**4**) could be the first chemical for studying this final step.

As a target, the DDD catalytic pocket is an excellent candidate for explaining the change in the mechanism of inhibition (drugs (**1**) to (**3**) *versus* (**4**)) and cross-activity of these drugs against HIV-1 integrase, because it carries the catalytic residues and also lies at the intersection of the two DNA substrates involved in the reaction. Compounds with a scaffold that is able to bind to this region might be capable of displaying several different inhibitory phenotypes, becoming specific inhibitors of complex formation, excision or strand transfer as a result of minor differences in their scaffold substitution.

On the other hand, differences in drug targets might also account for the different phenotypes of this family of compounds. Point mutations in the N-terminal domain of HIMAR1 (a MOS1 related *mariner* transposase) illustrate how changes in transposase-ITR interactions can have pleiotropic consequences. Some mutations completely destabilize the transposase-ITR complexes, whereas others allow the complexes to form, but inhibit the first or the second strand cleavage reaction [27]. This implies that compounds that target the N-terminal/ITR interaction could mirror the phenotype of N-terminal domain mutations. Identification of the target(s) of this family of compounds is already in progress, because it appears to be essential for further development, and for understanding the mechanism of action of these inhibitors in *Mos1* transposition and in the HIV-1 integration process.

Finally, we noted that bis-(heteroaryl)maleimide derivatives are new inhibitors of DDE-transposases, especially of *Mos1* transposase and HIV-1 integrase. Their identification may provide potent tools for studying the mechanism of *Mos1* transposition and/or for proposing new drugs that target HIV-1 integrase.

Cross Activity of DDE Enzyme Inhibitors

The ability of a drug to inhibit mechanistically-related enzymes (cross-activity) had already been illustrated by compounds that inhibit HIV-1 integrase, ASV and other related retroviral integrases [28]. Furthermore, diketo acid integrase inhibitors (5 CITEP and L-708,906) are also active against the distantly related RAG recombinases [29], although the activity of the drug is much lower (100 fold). More recently, screening for Tn*5* transposase inhibitors has been used to identify compounds that are active against HIV-1 integrase [20].

The work reported here constitutes a novel demonstration of cross-activity between Tn*5* and *Mos1* transposase inhibitors, and also, more interestingly, between MOS1 and HIV-1 integrase. The cross-activity of the new family of inhibitors we have identified indicates that MOS1 would provide an excellent surrogate model for an inhibitor screen. This surrogate model is very interesting for the following reasons. First, MOS1 transposition occurs in a eukaryotic environment, like the integrase, which makes it possible to envisage a test for inhibition that can be carried out in eukaryotic cells. Second, the structure of the catalytic domain of MOS1 is very similar to that of the retroviral RSV and HIV-1 integrases [9,10]. Third, the catalytic process of DNA cleavage looks similar to the processing reaction of retroviral integrase, the second strand cleavage reaction mimicking 3'-processing integrase activity (there is no inter-strand reaction implicating a hairpin intermediate in either of these reactions [19]). So far, the Tn*5* transpososome has been used as a model for the retroviral integrasome, but the *mariner* transpososome could constitute a new and probably better model of HIV-1 integrasome organization. Nevertheless, we should also note that these two models (*Mos1 versus* HIV-1) display

significant differences, such as the structure and arrangement of their DNA binding domains, the nature of the donor DNA substrate, and the DNA cleavage reaction (double strand versus single strand cleavage reaction) that mean that the two systems are not interchangeable. From this perspective, the use of cross-active drugs will provide an interesting tool for identifying similarities and differences between these two enzymes.

ABBREVIATIONS

IF = Inhibition factor

ITR = Inverted terminal repeat

LTR = Long terminal repeat

NTS = Non-transferred strand

RSV = Rous sarcoma virus

TS = Transferred strand

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